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TITLE: PARTIAL PURIFICATION AND CHARACTERIZATION OF PROTEASES IN TOBACCO LEAF AND CALLUS

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ABSTRACT: Ammonium sulfate fractionation, gel permeation and ion-exchange column chromatography were employed for partial purification of proteases from the leaf and callus of Samsun NN tobacco. The predominant protease in fully expanded leaves is sulfhydryl protease which can be activated by mercaptoethanol and EDTA and completely inhibited by iodoacetic acid but is unaffected by phenylmethane sulfonyl fluoride and pepstatin A. With hemoglobin and tobacco Fraction-1-protein as substrates, the proteases of tobacco leaves and calli had a pH optimum of 5. However, the specific activity of protease in calli was significantly higher than that in leaves. Reaction mixtures containing hemoglobin showed protease activity at least one-fold greater than that having Fraction-1-protein as the substrate. Casein was not an efficient substrate for tobacco protease. Three molecular weight variants of sulhydryl protease were separated by gel permeation column chromatography from the leaf protease fraction, however, there were only two variants in calli. The large molecular weight fraction of protease was rechromatographed on an ion-exchange column which further resolved it to three and two variants for leaf and callus tissues, respectively. The present results suggest that there are at least five variants of sulfhydryl protease in tobacco leaf, three variants in callus tissue, and that tobacco Fraction-1-protein can be metabolized by both leaf and callus sulfhydryl proteases.

REVIEW: The aim of this study was to identify the major proteases present in the Samsun NN cultivar of Oriental tobacco and to compare their physical and chemical characteristics. Leaf samples were taken about the time of flowering; callus tissue cultures were grown for about four weeks before extraction ana analysis. Centrifugation, precipitation, and dialysis (to remove potassium phosphate) were performed prior to chromatography. Four types of protease were identified, with sulfhydryl protease predominating. About twice as much protease was found in the calli as compared with the leaves. The authors also noted that much higher protease activity occurs in Samsun NN, Burley 21 and one of its variations, and in Nicotiana rustica than occurs in Nicotiana tabacum.

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